STUDIES IN THE PIGMENTARY SYSTEM OF CRUSTACEA

I. Color Changes and Diurnal Rhythm in Ligia baudiniana

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In the summer of 1936, while engaged in collecting near the biological station at St. George's West, I noticed distinct color differences in specimens of an isopod that was very common on the rocky ledges along the shore. The animals on the black porous rock above the high-tide mark were dark-grey or black, while those feeding on the algæ and other plant material covering the limestone of the intertidal zone were always yellowish-white in color. Closer observation led to the belief that this difference in coloration was due, not so much to morphological variations in pigment distribution, as to an active physiological concentration and dispersion of pigment within cells. It was therefore decided to investigate more fully the chromatophoral behavior in these isopods which were identified as *Ligia baudiniana*.

The mechanism of chromatic change in the isopod crustaceans has not been definitely established. Following the early studies of Pouchet (1876) on the color changes of decapod crustaceans, Matzdorff (1883) published a report on the coloration of *Idotea*. He was of the opinion that chromatophoral activity was under the control of the nervous system, but decided that the inconclusive results obtained by severing the ventral nerve cord were to be attributed to injury brought about by the operative manipulation. Menke (1911), in a study of the rhythmic activity of color changes in isopods, believed that the mechanism for such responses was based upon physiologically innervated melanophores, referring in support of this to a figure of an innervated chromatophore from the integument of a young *Philoscia* in Weber's (1881) paper. It appears from a study of the figure that the nerve fiber supplying the chromatophore is a process of a peripheral nerve cell, the other processes of which supply the sensory (?) hairs on the body. The relationship, however, is not very evident and no further anatomical detail is given. The implication drawn from the illustration

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was that chromatophoral activity depended upon a local receptor-effector mechanism, a condition which subsequent study of blinded animals failed to confirm (Tait, 1910). Menke reported that section of the ventral nerve cord had no effect on metachrosis, but that the chromatophores became dispersed if the dorsal side of a segment were cut without damaging the dorsal vessel.

Several years later, Perkins (1928) and Koller (1928) showed that pigmentary changes in the decapod crustaceans were under the control of an endocrine substance that had its origin in the eye-stalks. When appropriate extracts of the eye-stalks of *Palæmonetes* were injected into shrimps which had become dark as a consequence of their having been kept upon a black background, the animals soon became light due to a concentration of pigment within the integumentary chromatophores. Perkins' studies on *Palæmonetes* were confirmed and extended by Brown (1935), who believes that the chromatic components of the pigmentary system are controlled by separate hormones.

Extensive experiments by Kropp and Perkins (1933) and by Hanström (1935) established the presence of a humoral chromatophore activator in the eye-stalks of a wide variety of crustaceans. Hanström, in addition, has located two organs suspected of glandular function in the eye-stalks of many decapods, and one of these, the blood gland (*Blütdruse*), he believes chiefly responsible for the control of pigmentary activity. In some crustaceans, *Gebia affinis* and *Hippa talpoida,* these glands are present, not within the eye-stalk, but in the head on the surface of the brain. This was confirmed by experiments which showed that extracts of the eye-stalks of these two crustaceans when injected into blinded *Palæmonetes,* had no effect upon the dispersed chromatophores, while extracts of the heads were as active in effecting concentration of the pigment cells as were preparations from the eye-stalks of *Palæmonetes.*

In view of these advances in the study of color changes of decapod crustaceans, it was thought advisable to study melanophore activity in the isopods with regard to endocrine control.

Materials and Methods

Specimens were collected along the shore where the isopods are found in large numbers, feeding upon the plant material that is uncovered by the ebbing tide. The animals were obtained by lifting rocks and dropping them into a wooden bucket, the impact being sufficient to jar the individuals loose from the under sides of the stones. Collections were made daily to insure a supply of normal, active individuals.

In following the responses to changes in color of background, a large white porcelain dish, the bottom of which was covered with moist white sand, served as a white background. For adaptation to black backgrounds a glass bowl, the outside of which had been covered with black paint, was used, the sand for the bottom of this vessel being mixed with an equal amount of pulverized coal. The containers were illuminated by light from a 60-watt lamp at a distance of 18 inches.

It became necessary during the course of these experiments to observe the reactions of blinded *Ligia*. Blinding by extirpation of the sessile eyes was unsatisfactory because the ensuing hemorrhage invariably caused the death of operated individuals. Such operations were eventually abandoned and blinding was accomplished by covering the eyes with an opaque mass, obtained by mixing plaster of Paris and lampblack with a little water. This mixture was applied over the head so that the eyes were completely covered, and, after being allowed to dry, was coated with a thin layer of waterproof paste to prevent moistening and crumbling.

Extracts of the heads of *Ligia* were prepared in various concentrations to determine the possibility of an endocrine factor in pigmentary changes. These were prepared in two ways: in one, the heads were crushed and ground with 1.0 cc. of sea-water in a mortar, most of the coarse detritus was separated off, and the remaining fluid drawn directly into a hypodermic syringe for injection; the second method was essentially the same, except that the triturated heads were transferred to a test tube and brought to a boil. The heat was sufficient to clump most of the solid material so that the supernatant fluid was almost water-clear. The solution was allowed to cool and was then drawn into the syringe.

In the experiments where such extracts were injected, the pigmentary condition of the specimen was first examined by means of a dissecting microscope, and observations were again made within 10 minutes after treatment. Care was taken, when injecting, to insert the needle dorsally into the body spaces, well anterior to the heart (usually between the fifth and sixth thoracic segments), to avoid loss of body fluid. Control injections consisted of both boiled and ordinary sea water.

Color Pattern

The dominant and most obvious component of the chromotophore system in *Ligia* consists of cells containing a black pigment, possibly a melanin. These pigment cells are distributed over the entire surface of the animal, being more numerous and apparently smaller in size

on the dorsal side, especially in the region of the mid-line; they are less densely aggregated near the lateral margins of the tergites (Fig. 2).

A second component of this system consists of white pigment. This occurs in many individuals as rather large clusters on the posterior, dorsal surface, and, by examination with the low powers of the dissecting microscope, does not appear cellular. In addition to massed

TABLE I

Responses of the melanophores of Ligia baudiniana to changes in background.

Series I

Time after transfer of 7 dark specimens to a white background	Condition of melanophores			
	0	A	B	C
5 minutes		7		
10 minutes		1	6	
20 minutes			2	4
35 minutes			1	5

Series II

Time after transfer of above white specimens to a black background	0	A	B	C
17 minutes		1	5	
95 minutes	2		4	
240 minutes	4	1	1	
300 minutes	6			

Series III

Time after transfer of 7 dark specimens to a white background	0	A	B	C
5 minutes		7		
30 minutes			4	3

pigment there are, however, definite cells containing this white substance. Such "guanophores" appear to show a limited activity in the concentration and dispersion of their pigment, but close observation of their behavior was not undertaken in this study. A yellow pigment of some sort is also present and is most noticeable in preserved specimens. This combination of body colors is very effective in maintaining a concealing coloration of the animals in their native habitat.

Table II

Responses of Ligia to injection of extracts. The concentration per cc. designates the number of heads triturated in 1.0 cc. of sea-water. E_w, extract prepared from white-adapted specimens. E_b, extract prepared from black-adapted *Ligia*; A_b, black-adapted isopods were injected; A_w, white-adapted animals were injected. In Series I, the extracts were prepared from background-adapted animals, in Series II extracts were similarly prepared and boiled. Extracts for Series III were prepared unboiled, from specimens during the two conditions of diurnal activity, while in Series IV similar extracts were boiled previous to injection.

Series I

Concentration per cc.	E_w into A_b					E_b into A_b					E_b into A_w				
	No. of Ligia inject.	0	A	B	C	No. of Ligia inject.	0	A	B	C	No. of Ligia inject.	0	A	B	C
4.....	11	2	3	6											
4.....	10	2*	3	5											
4.....	9		4	5											
4.....	10	1	1	8											
8.....	10	1	4	5											
4.....						14	1		13		3				3
Total.....	50	4	15	29		14	1		13		3				3

Series II

25.....	3			3											
8.....	5		1	3	1										
4.....	10			2	8										
10.....						4			4		4				4
Total.....	18		1	8	9	4			4		4				4

Series III

4.....	11			11											
4.....	10			10											
4.....						8	7	1							
4.....						8	3	5							
4.....						14	1	5	8						
4.....						5	1		4						
Total	21			21		35	12	11	12						

TABLE II (cont.)

Series IV

Concentration per cc.	E_w into A_b					E_b into A_b					E_b into A_w				
	No. of Ligia inject.	0	A	B	C	No. of Ligia inject.	0	A	B	C	No. of Ligia inject.	0	A	B	C
10.....	4				4										
5.....	4			4											
2.5.....	4			1	3										
1.25.....	4			1	3										
10.....						4			1	3					
5.....						4			1	3					
2.5.....						6	2	3	1*						
10.....						4				4					
5.....						4		1	3						
2.5.....						4		2	2						
1.25.....						4			4						
4.....						4	1*	1*		2	4				4
2.....						4		1	2	1					
1.....						4	1*		1	2					
0.5.....						4		2	2						
0.25.....						4		4							
0.12.....						4		3	1						
0.06.....						5	1	3	1						
4.....						8	2*		2	4					
Total.....	16			6	10	67	3	19	20	19	4				4

* Specimen died.

COLOR ADAPTATIONS TO BACKGROUNDS AND EFFECTS OF BLINDING

The surmise that there was a physiological color change in adaptation to the color of the background was confirmed by testing the responses of specimens in the laboratory on black and on white backgrounds (Table I, and Figs. 2 and 3). The melanophore changes of the black-adapted *Ligia* of Series I and Series III were recorded after the animals were transferred to a white background. The specimens of Series II were those of Series I which had become adapted to the white background and were then transferred to the black vessel as a converse experiment. The conditions of the melanophores recorded in Tables I and II are designated by the symbols used in Fig. 1. The color changes

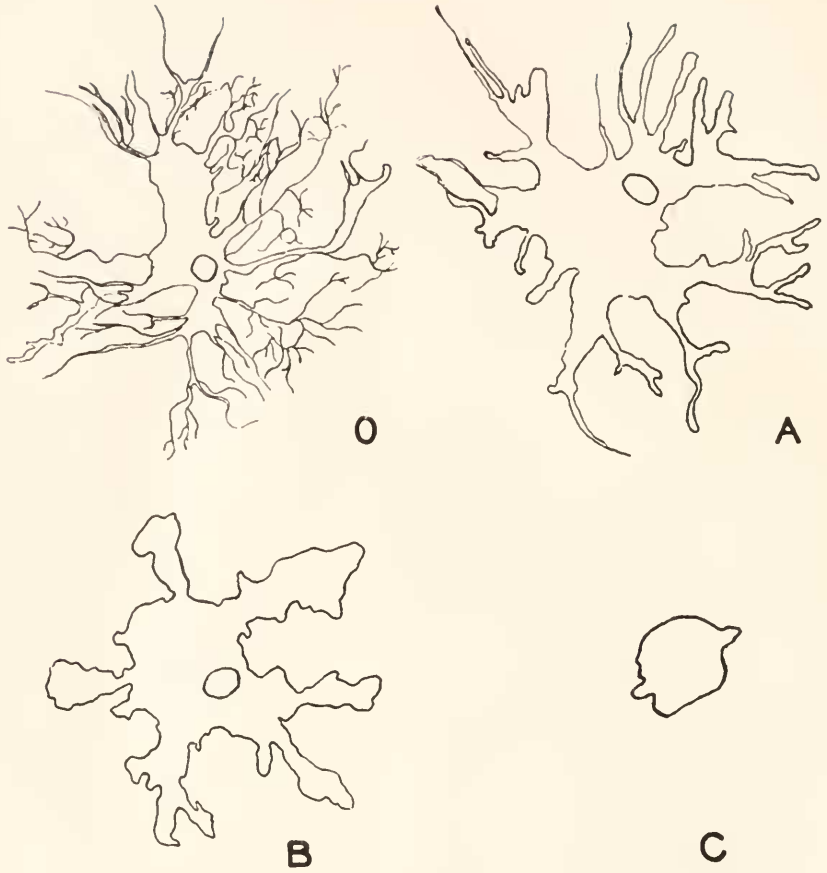

FIG. 1. Outline drawings of melanophores, showing the conditions of the cells in four stages from dispersion to concentration of pigment. *O*, the maximally dispersed state, with many delicate processes visible; *A*, beginning of concentration, the distal processes of the cells losing their delicate tracery, and ending in blunt rounded knobs; *B*, the stellate condition; *C*, the punctate state, with the nucleus obscured by the pigment granules.

in response to background are seen (Table I) to be more rapid during concentration than during dispersion, being in this respect similar to the behavior of the chromatophores in *Palæmonetes*.

It became desirable after these preliminary experiments to observe the color reactions of blinded *Ligia*. For the isopod crustaceans, Tait (1910) reported darkening of *Ligia occanica* when the eyes were covered with an opaque mass, and Piéron (1914) obtained similar results with blinded *Idotea*. In the decapods, however, differing types of responses have been found, depending upon the method of blinding em-

ployed by the investigator, and upon the crustacean studied. In operating upon the pedunculate eyes of the decapods, two methods of blinding are possible. One procedure is to remove or destroy the retina only; the second method is to excise the entire eye-stalk, thereby removing Hanström's blood-gland in addition to the retina. The pigmentary changes in the animal resulting from the second type of operation are due more to deficiency of the chromatophorotropic hormones from the blood stream than to destruction of the retina.

With *Palæmonetes,* which does not possess a melanophore system in the body pigment, Brown found that cauterization of the retina resulted in a loose concentration of the red pigment and a half-way dispersed condition of the yellow pigment cells. Ablation of both eye-stalks, however, effected a full dispersion of the red and the yellow chromatophores and random variations in the white pigment cells. Carlson (1935, 1936) in studying *Uca pugilator,* which possesses melanophores in the pigmentary system of its integument, found that removal of the distal thirds of both eye-stalks had no effect on the pigment cells, whereas total removal of both stalks (the blood-gland is located in the middle third of the eye-stalk) caused a permanent concentration of the melanophores and the erythrophores, while "the yellow chromatophores were slightly more contracted than the stellate state, and the white ones a little more expanded than that state." Abramowitz (1935) also reported concentration of the black pigment cells and dispersion of the guanophores of *Portunus anceps,* following total removal of the eye-stalks. The pigmentary reactions of the two brachyurans to these operations parallel the color changes of many of the lower vertebrates after hypophysectomy. There is a striking similarity in physiological effect between crustacean eye-stalk extract and the melanophore-dispersing principle of the vertebrate hypophysis, as reported in recent studies by Abramowitz (1936*a,* 1936*b*).

In preliminary tests a number of *Ligia* were blinded by removal of the eyes with a spear-point needle. Such specimens, with their melanophores initially punctate, became darker after the operation, but it was thought advisable to repeat the experiment, using a technique that involved less injury to the animal. Similar results were obtained when 10 white-adapted *Ligia* were blinded by covering the eyes with an opaque mass. The melanophores of all 10 isopods became maximally dispersed within an hour; five minutes after this last observation (3:45 P.M.) the specimens were placed in the dark-room, and when examined later in the evening (5:30 P.M.) 4 of them were light, 3 were intermediate, and 3 were still dark. The next morning (at 8:00 A.M.) of 5 surviving animals, 4 were dark and 1 was intermediate in color.

These confusing results became more intelligible when further observations showed that the isopods underwent a diurnal rhythm in melanophore activity in constant darkness. Under such conditions the pigment in the black cells was dispersed during the day and concentrated at night. Upon an illuminated black background, however, the rhythm did not appear at night; the isopods remained dark. The following notes on a series of animals in the dark-room indicate the pigmentary condition of the isopods:

June 23 10:30 P.M. Six white *Ligia* with melanophores punctate were placed in the dark-room.
June 24 9:00 A.M. Two specimens are still light; 4 are dark with the melanophores dispersed.
June 24 6:30 P.M. Same as at 9:00 A.M.
June 24 10:45 P.M. Six specimens are light: in 3 the melanophores are punctate, while in the remaining 3 they are punctate and stellate.
June 25 10:15 A.M. All specimens are dark: 4 with melanophores maximally dispersed; 2 show them stellate and slightly more dispersed.
June 26 12:30 A.M. All 6 isopods are light with the melanophores punctate.
June 26 10:30 A.M. Six specimens are dark.
June 26 4:30 P.M. Same as at 10:30 A.M.
June 26 10:15 P.M. All the animals are light.

Unfortunately the critical times during the day when these rhythmic changes were initiated could not be determined. It became evident that considerable variation existed in the onset of the changes, and that such variation might be due to the effects of captivity. There could, however, be no doubt of the existence of a pigmentary rhythm, since it was observed repeatedly both under laboratory conditions and at night in the natural habitat of the isopods.

Effects of Injecting Extracts

Hanström (1935) showed that the activity of crustacean eye-stalk extracts in concentrating the dispersed chromatophores of blinded *Palæmonetes* was correlated with the presence of the blood-gland in the

EXPLANATION OF PLATE I

Fig. 2. On the left is a specimen that has been darkened by exposure to a black background; on the right an isopod adapted to a white background. The photograph was taken from *Ligia* which had been killed with hot water and preserved in formalin.

Fig. 3. Appendages from white-adapted and black-adapted individuals, showing the two extreme conditions of the melanophores.

PLATE I

eye-stalks used. Stalk extracts from the eyes of *Gebia affinis* and *Hippa talpoida* were ineffective in concentrating dispersed chromatophores because the blood-gland is absent from the eye-stalks of these crustaceans. Extracts prepared from the heads of these two decapods are active because the organs presumed to be of endocrine function are located on the surface of the brain. Since the eyes of *Ligia* are sessile, entire heads of specimens were used in preparing extracts. The bodily changes in color following the injection of extracts prepared from specimens in different pigmentary conditions are indicated in Table II.

It is evident that black-adapted specimens responded to such treatment by a concentration of their melanophores, while white-adapted isopods showed no change. Control injections of sea-water into 42 black-adapted *Ligia* were without effect on the dispersed melanophores of 37 individuals, while 5 animals became perceptibly lighter in color. These 5 specimens were part of a group of 14 isopods that were injected at night (11:15–11:45 P.M.), a time when specimens in the dark-room are light because of the diurnal periodicity.

The behavior of the black pigment cells, following injection of the extract into *Ligia*, is in striking contrast to that of the melanophores of *Uca*. Carlson (1936) and Abramowitz (1936*b*) have shown that the dark coloration may be restored to blinded *Uca* by the injection of eye-stalk extracts. The responses of the melanophores of the two brachyurans when compared with the diametrically opposite behavior of those in *Ligia* may be due to some fundamental difference in the nature of the pigment cells (*vide*, Bigney, 1919, on the responses of retinal and body pigments of frogs to adrenalin), or it may be due to the existence of two different hormones, one causing concentration and the other effecting dispersion of the black pigment. Unfortunately, critical experiments to decide this second possibility were not performed.

The phenomenon of a diurnal rhythm in the activity of the chromatophores and retinal pigments of crustaceans is now well known through the work of Keeble and Gamble, Menke, Piéron, and Welsh. The basis for this activity is, however, less well understood. Piéron believed the color changes of *Idotea* to be due to a nervous mechanism, and said of the rhythmic changes, "the nervous centers can periodically control the reflex without being directly stimulated by the sensory impressions (received by the eyes)." In view of recent developments showing that the pigmentary activities of the body and the retina are under hormonal influence (Perkins, 1928; Kleinholz, 1936), Piéron's explanation should be revised to allow for the humoral factor.

Several interesting speculations as to the basis for this diurnal activity have been put forward by Welsh (1936) and by myself. Welsh

suggests, "There may be a rhythmic secretory cycle in the gland which continues under constant conditions or the situation may be much more complex and the rhythm in the eye may only accompany a general rhythmic activity which results from a series of changes involving the nervous-endocrine systems." A third possibility, supplementary to the first suggestion, is that the rhythm may be due to a diurnal cycle of exhaustion and elaboration of the secretory material when the animal is maintained under constant conditions.

Physiological tests fail to substantiate this last possibility. Examination of the data in Table II shows that extracts prepared from specimens of *Ligia* in the two diurnal pigmentary conditions are practically equally effective in causing concentration of the dispersed pigment in the melanophores. The greater activity of boiled extracts has been reported in similar observations by Perkins and Snook (1931) and by Hanström (1935).

There is as yet no direct evidence favoring either of the two remaining possibilities. More complicated reactions than are at present indicated may be involved in such periodic pigmentary changes. While neither of these hypotheses really clarifies the means by which the rhythmic activity originates, such assumptions are of assistance in narrowing down the number of systems to be studied in the hope that eventually more light may be thrown upon the nature of this phenomenon.

Summary

1. The bodily changes in color of *Ligia baudiniana* upon black and upon white backgrounds are due chiefly to a dispersion and concentration of pigment granules within melanophores.

2. When the animals are kept in constant darkness, there is a diurnal rhythm in pigmentary activity, the isopods being dark during the day, and light at night.

3. Injection of aqueous extracts of heads into the body spaces of dark *Ligia* brings about lightening in color by a concentration of the melanophores.

4. Extracts from the heads of dark and of light specimens in the two conditions of diurnal rhythm are practically equally effective in concentrating the melanophores of dark isopods. It may be concluded from this that the diurnal pigmentary activity is not due to a cycle of exhaustion and elaboration of secretory material in the endocrine gland controlling the color changes.

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